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The trace elements chromium, manganese, copper and zinc in fetal liver tissue

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1 Introduction

It has been known for some time that fetal liver tissue contains considerably more copper than adult liver tissue and more than any other tissue [10, 34, 44, 47]. A major portion of the metal is bound to two specific proteins. One of these has been designated as neonatal hepatic mitochondrocuprein, the other is metallothionein which is located in the cytosol [35, 36, 37, 47]. It appears appropriate to assume a storage function of the fetal liver tissue for copper because the elevated copper concentrations decrease to adult levels within a few weeks after birth. More recently, a similar phenomenon has been described for zinc, chromium, and manganese with variable results [12, 13, 22, 47].

It must be assumed that the analysis of trace elements in animal tissue is influenced by the variation in processing and analytical methods as well as metal loss and contamination. Therefore, we set out to evaluate these reports with a combination of modern methods and to verify the result with reference material and strict laboratory standards [9].

2 Material and methods

2.1 Experimental animals

We used 41 pregnant white New Zealand rabbits from a commercial supplier (GRÄFLICH DEGENFELD-SCHOMBURG'SCHES RENTAMT, Geislingen/Steige). The animals were kept in wire cages in fully air-conditioned rooms at a relative humidity of 50–60%. They were fed ad libitum with commercial rabbit foot (ZUCHT-PELLETS, DEUTSCHE KRAFTFUTTER GmbH, Worms). They drank water from the local water supply. The mean weight of the pregnant animals was 4.26 ± 0.37 kg.

2.2 Experimental material

The does were killed on the 28th or 29th postconceptional day by intravenous injection of 1.5 g Phenobarbital. The liver was removed and exsanguinated. The vascular system of the liver was not perfused. The liver surface was rinsed off with demineralized water and a tissue sample was removed

Curriculum vitae

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from the liver margin which is poor in connective tissue. This was kept for a few minutes in a humidified plastic Petri dish until processing.
Three fetuses were removed from the uterus at random, they were decapitated and exsanguinated. The fetal livers were cleansed and preserved as described above.

2.3 Reference material

We used for control of accuracy the reference material "1577 Bovine Liver" from the U.S. National Bureau of Standards (U.S. DEPARTMENT OF COMMERCE, Washington, D.C.). It consists of pooled lyophilized beef liver tissue. Mean and standard deviation of the concentrations of different metals measured with various methods were used as reference value.

2.4 Analysis

2.4.1 Reagents

Demineralized water used in rinsing glassware and for the dilution of reagents and samples was regularly assayed for contamination at the highest sensitivity of the analytical apparatus. 65% nitric acid (Suprapur specific weight 1.40; MERCK COMPANY, Darmstadt) and 25% analytical ammonia (specific weight 0.91, MERCK COMPANY, Darmstadt) were used in processing. Since neither reagent could be checked directly for contamination we neutralized 1 ml nitric acid with 1.4 ml ammonia and analysed this ammonia nitrate solution.
Calibration solutions of the metals to be analysed were made from concentrates (RIEDL DE HAEN COMPANY, Hannover). They contained 1.92 mMol/l chromium; 1.82 mMol/l manganese; 1.57 mMol/l copper and 1.53 mMol/l zinc (corresponding to 100 mg/l each). From these master solutions those used in the assays were made fresh daily.

2.4.2 Processing

For the processing of the liver tissue we used the special apparatus of Chemie-Analytic-Vertrieb, Weinheim, Teck. It consists of a heating block which admits 10 pressure containers within each of which there is one polytetrafluorethylen (PTFE) vessel. The lid of this vessel is closed with a spring at 50 kp/mm [23]. The PTFE vessels were boiled for 15–20 minutes in 30% nitric acid and subsequently rinsed several times with demineralized water. 0.2–0.4 g of fresh liver tissue were weighed into each PTFE vessel and 0.4–0.6 ml of nitric acid was added. This material was heated in the pressure vessel for two hours at 410°K. The liquid yellow-green clear residue was brought with ammonia to a pH of 6–7.5. From this material samples for analysis were made by dilution with demineralized water. This step is necessary so that the atomic absorption spectrophotometry can be operated in the linear range. Dilution for the chromium assay was 1:3, for manganese 1:20 and for copper and zinc 1:100. With each processing batch a sample of the reference material (100 mg weight; 0.5 ml acid) and a blank containing only acid in the PTFE vessel was analyzed.

2.4.3 Analytical method

For trace analysis we used the atomic absorption spectrophotometer of PERKIN ELMER COMPANY, Überlingen with a 3-slit mixed chamber burner for zinc determination and a graphite tube cuvette HGA 72 for the determination of chromium, manganese, and copper. All measurements used background compensation using either deuterium or a halogen lamp as radiation source depending on the wave lengths.
The zinc assay was done with an air-acethylen flame. The diluted residue was directly suctioned into the apparatus and compared with the extinction values of the calibrating solutions (1.53–15.3 μMol/l).
For chromium, manganese, and copper analyses the diluted residue was mixed with 3 or 4 standard solutions at 1:2 and these mixtures were examined in the graphite tube cuvette. The extinction values were then plotted over the added concentrations; this resulted in a linear addition curve and the analytical value could be determined graphically or mathematically. With the choice of appropriate temperature-time correlations for the graphite tube cuvette a disturbing smoke development during atomization could be avoided (Tab. I).

Tab. I. Temperature-time settings of HGA 72. For technical reasons the atomization temperature for the chromium analysis was set low.

	duration in sec			temperature (°K)		
	Cr	Mn	Cu	Cr	Mn	Cu
Drying	30	30	30	370	370	370
Pre-heating	15	15	15	620	470	570
Continuous increase in temperature				1650	1380	1170
Maintenance of temperature	15	15	5	1650	1380	1170
Atomization				2070	2870	2870

2.5 Statistics

The frequency distributions of blanks and measured values in maternal and fetal liver tissue were plotted as the upper class limits of the sum frequencies as ordinates in the probability grid with linear or logarithmic abscissa. In order to characterize log-normal distributions medians and standard deviations were computed [20]. The latter is the value with which the median has to be multiplied or divided in order to define a 1s, 2s, or 3s range.

For the comparison of the maternal and fetal manganese concentration the one-tailed t-test for comparison of means was used (normal distribution of transformed values, stable variants). The similar comparisons of chromium values used the distribution-free Wilcoxon test. Regression analysis (Cu, Zn) used for the zinc concentration the measured original values and for the copper concentration the transformed values (normal distribution).

The analytical limits of a method are determined by the blanks. The processing of the samples can easily lead to contamination. With the methods described and the metals analyzed losses are less probable. From at least 20 blanks (x_{B1}) the analytical limit $\underline{X}$ is derived from the formula: $\underline{X} = \bar{x}_{B1} + 3s_{B1}$, i.e. only a sample value above the upper 3s range of the blanks can assumed as being true. The upper 6s range of the blank value is the proof limit for accuracy. Thus, if the value is in the analytical limits it may be assumed with certainty that the true value of the proof limit for accuracy is not exceeded [21,41]. In calculating the analytical limits and proof limits for accuracy it is assumed that the blanks are normally distributed. However, this has to be verified in each case and is in trace element analysis often not true. The analytical limits of log normal blank values must be defined differently. In this case we only indicate the range of the single values.

3 Results

3.1 Blank values and analytical limits

For manganese the analytical limit was calculated as described above. It was 0.12 µMol/liter.

For zinc and copper no background signal was obtained from the neutralized HNO_3 in the blank at a dilution of 1:10. The analytical limits here are so low that it is irrelevant for our measurements. The blanks of the chromium analysis are log-normally distributed. The lowest value was 19.4 nMol/liter, the highest 635 nMol/liter.

3.2 Comparison with reference material

Reference values and the values found by us in the reference material "1577 Bovine Liver" are given in Tab. II.

Tab. II. Comparison with reference material.

Element	Reference Value ($\bar{x} \pm 1s$)	Own Measurement ($\bar{x} \pm 1s$)
Zn	2.0 ± 0.2 mMol/kg	2.0 ± 0.3 mMol/kg (n = 16)
Cu	30.4 ± 1.5 mMol/kg	27.6 ± 2.8 mMol/kg (n = 14)
Mn	187.5 ± 18.2 µMol/kg	194.1 ± 42.8 µMol/kg (n = 21)

For methodical reasons a reference value for chromium is not indicated. Inofficially a concentration of 3.8 µMol/kg is given. In 8 samples of the reference material we found concentrations between 3.2 and 5 µMol/kg.

3.3 Accuracy

The day-to-day as well as serial accuracy is given in Tab. III. (Measurements refer only to atomic absorption spectrophotometry).

Tab. III. Accuracy.

Element	Day-to-Day Accuracy	Serial Accuracy
Cr	14.99%	5.1; 13.8; 17.9%
Mn	4.90%	4.5; 4.1; 2.8; 1.9; 3.5; 2.2%
Cu	4.54%	6.5; 6.8; 6.9; 6.9; 9.7; 9.7%
Zn	4.40%	1.7; 1.7; 2.1; 1.3; 2.8; 1.8%

3.4 Zinc

Zinc concentrations found by us in maternal and fetal liver tissue are approximately normally distributed (Fig. 1). Both distributions differ in the central tendency and the variability. (Tab. IV). Depending on the choice of medians or means the fetal zinc concentrations are 3.7 and 3.6 times higher than the respective maternal concentrations.

There is a positive correlation between maternal and fetal zinc concentration in the liver tissue ($\alpha = 0.05$, Fig. 2).

3.5 Copper

The concentration of copper in maternal and fetal liver tissue is log-normally distributed (Fig. 3). The values of the central tendency and the variability are shown in Tab. V.

Copper concentrations in fetal liver tissue are on the average 5.5 times higher than in the maternal liver tissue. A correlation between copper concentration in the maternal liver tissue and the copper concentration in the fetal liver tissue was not found. However, we found a positive correlation between zinc and copper concentrations in maternal liver tissue ($\alpha = 0.01$). With an increasing zinc concentration the copper concentration in the liver tissue increases (Fig. 4). There was no correlation between the corresponding fetal values.

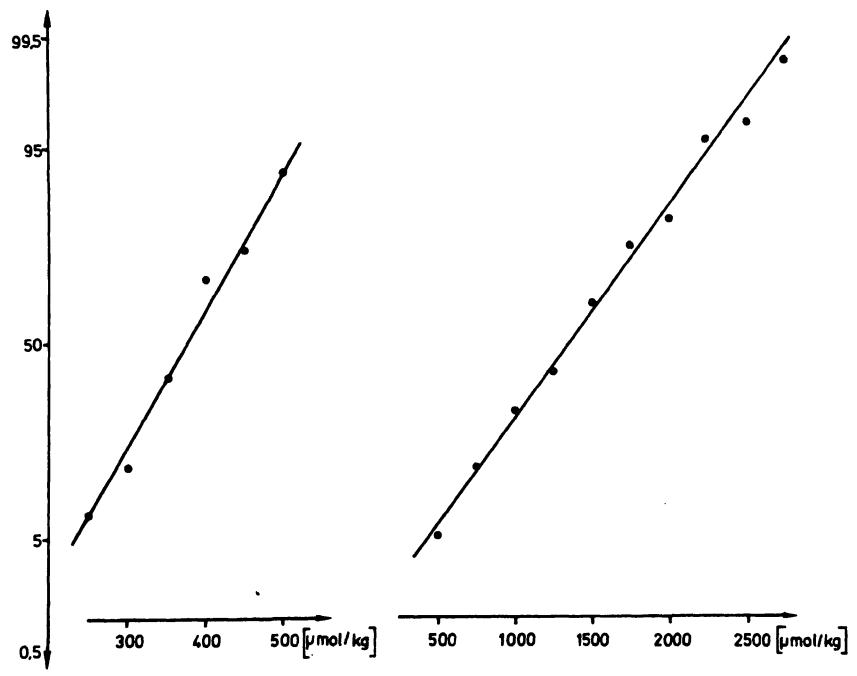

Fig. 1. Zinc concentrations of mothers (left) and fetuses (right) in a probability grid. Ordinates are the sum frequencies of the upper class limits.

Tab. IV. Zinc in maternal and fetal liver tissue. Parameter of central tendency and variability. Concentrations of μMol/kg wet weight.

Sample	n	$x_{i_{min}}$	$x_{i_{max}}$	median	mean	standard deviation
maternal liver tissue	41	203	550	361	373	78.84
fetal liver tissue	118	241	2754	1343	1348	539.04

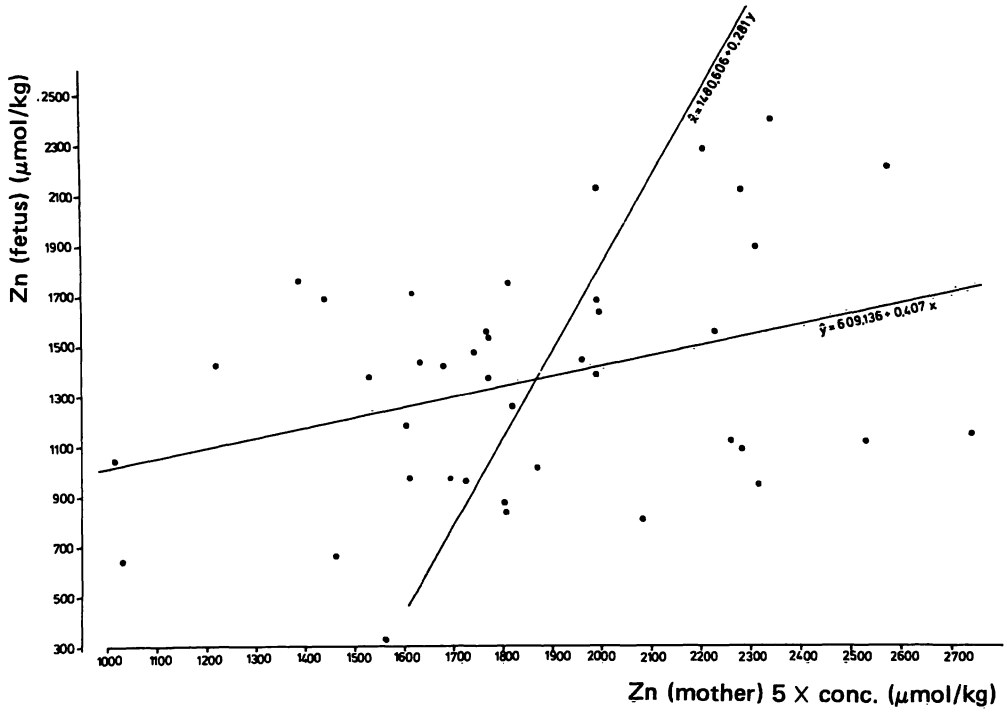

Fig. 2. Regression between zinc concentrations in maternal and fetal liver tissue.

Tab. V. Copper in maternal and fetal liver tissue. Parameter of central tendency and variability. Concentrations in μMol/kg wet weight. Because for log-normal distributions 1s and 2s ranges about the median are given, the mean has not been indicated.

Sample	n	$x_{i_{min}}$	$x_{i_{max}}$	median	standard deviation factor	
maternal liver tissue	35	48	157	72.3	1s range	1.37
					2s range	1.89
fetal liver tissue	100	116	766	396.3	1s range	1.36
					2s range	1.85

3.6 Manganese

As for copper, the manganese concentrations in the maternal and fetal liver tissue are log-normally distributed (Fig. 5). Central tendency and variability of the samples is indicated in Tab. VI.

The manganese concentration in maternal liver tissue is greater than in the fetal liver ($\alpha = 0.01$).

3.7 Chromium

Chromium concentrations in fetuses as well as in mothers showed a pronounced left peak with a skew towards the right side. None of the transformation procedures yielded a normal distribution. Therefore, we cite single measured values for the distribution (Tab. VII).

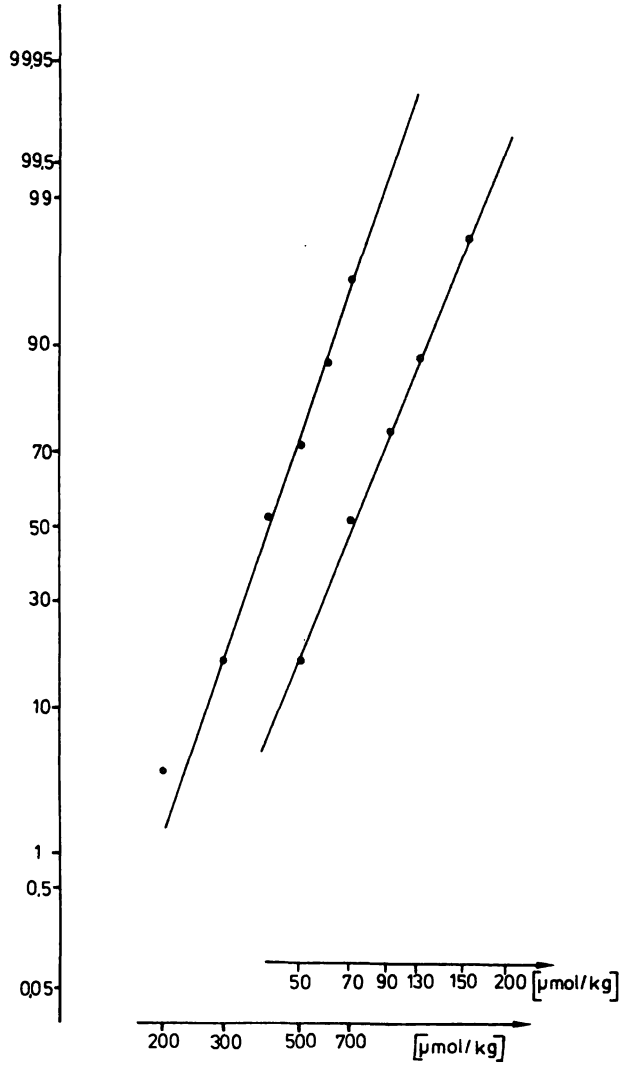

Fig. 3. Copper concentrations in a probability grid with logarithmic abscissa. Ordinates are the sum frequencies of the upper class limits (fetuses on the left, mothers on the right).

The mean chromium concentration in fetal liver tissue is not significantly different from the maternal values.

4 Discussion

4.1 Methods

When using atomic absorption spectrophotometry for trace element analysis of non-liquid organic materials it is necessary to pre-process the sample. The method used by us has the advantage of low losses [4, 6, 7, 23]. With elements in very low concentrations there is a risk of contamination [29, 43]. Single determinations of the metals in the reference material demonstrate that the entire analytic sequence has a large scatter. The accuracy demanded in conventional analyses has not yet been achieved in the trace analysis of metalic elements in animal and plant tissue [9, 29]. This is evident from the blank values. Methodical and statistical errors may yield a measurement even if the element in question is not even present in the material [41, 43].

For the chromium determination the blank values were log-normally distributed. The highest blank values overlapped with the lowest values in the liver tissue. Thus, the interpretation of chromium concentrations must be made with caution.

4.2 The trace elements Cu, Zn, Mn, and Cr in maternal and fetal liver tissue.

In the present paper, we examined metals which are essential trace elements in animals. The biological significance of these elements cannot be discussed in detail here. For this we refere to other reviews [10, 11, 12, 26, 27, 28, 39, 44].

4.2.1 Copper and zinc

It has been known for some time that the neonatal liver has a higher copper concentration than that of the mother. With few exceptions most mammals

Tab. VI. Manganese in maternal and fetal liver tissue. Parameter of central tendency and variability. Concentrations in µMol/kg wet weight.

Sample	n	xi_{min}	xi_{max}	median	standard deviation factor	
maternal liver tissue	41	17.3	89.0	36.4	1 s range	1.49
					2 s range	2.22
fetal liver tissue	118	2.9	70.8	22.7	1 s range	1.52
					2 s range	2.32

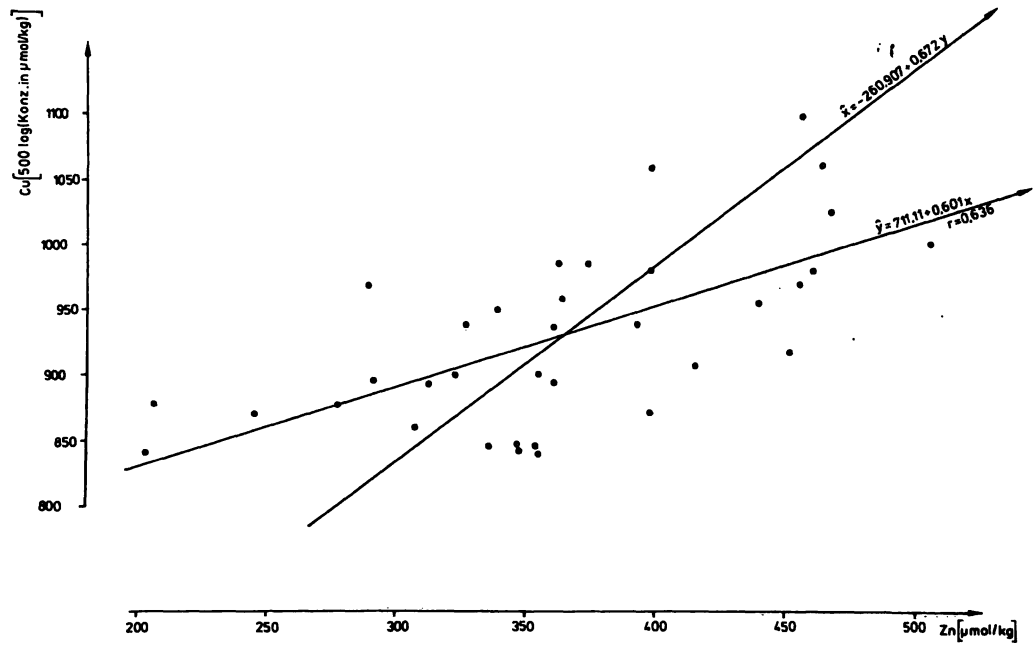

Fig. 4. Regression between zinc and copper concentrations in maternal liver tissue.

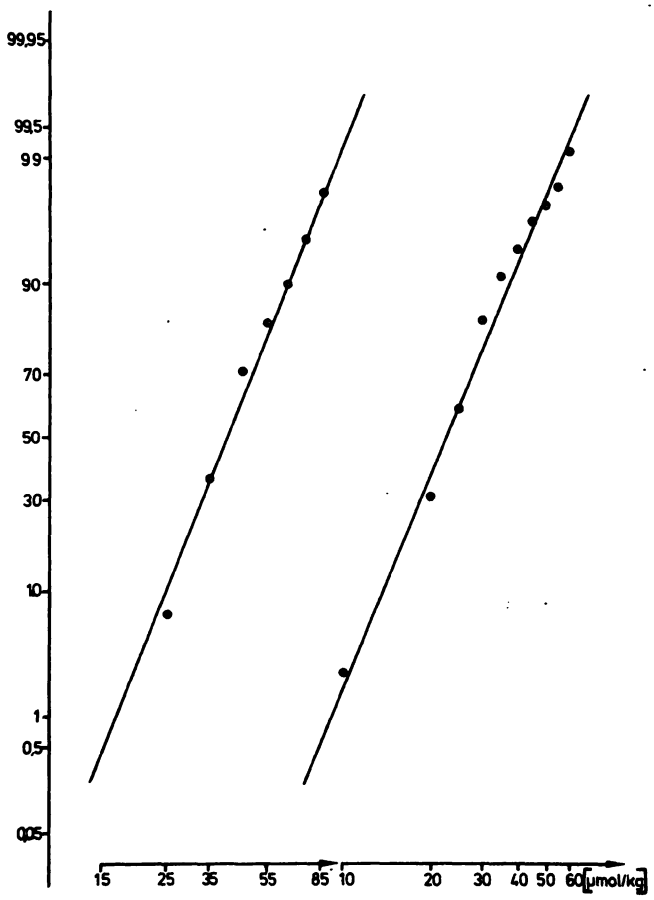

Fig. 5. Manganese concentrations in maternal (left) and fetal (right) liver tissue in a probability grid with logarithmic abscissa.

demonstrate an increase in liver copper concentration during fetal development [10, 34, 44, 47]. **The liver is the principal organ for copper turnover.** Copper which has been absorbed or introduced transplacentally is bound to albumin and transferred into the liver. Copper not needed for hepatic enzyme synthesis (superoxide dismutase, cytochromoxidase, monoaminoxidase, etc) is excreted with the bile or used for ceruloplasmin synthesis [10]. The excretory function of the fetal liver for excess copper and ceruloplasmin synthesis are limited. Copper is retained in the fetal liver. Free copper ions are extremely toxic and thus must be bound to decontaminating proteins [46]. In adults this is accomplished by metalothionein, a soluble cytoplasmic protein, rich in sulfhydryl groups; it also binds zinc, copper, and mercury. This protein is present in the fetal liver. Metallothionein capacity is insufficient in patients with Wilson's disease where there is a disturbance in the copper utilization for ceruloplasmin synthesis is underdeveloped. In order to protect the organism from the effect of free copper ions an insoluble mitochondrial cuprein, is formed as an additional binding protein [10, 35, 36, 37]. This has also been found in rats who were given toxic amounts of copper in their drinking water [33].

Tab. VII. Chromium in maternal and fetal liver tissue. Parameter of central tendency and variability. Concentration in µMol/kg wet weight.

Sample	n	$x_{i_{min}}$	$x_{i_{max}}$	median	model class
maternal liver tissue	38	0.23	7.49	1.30	1.00–1.49
fetal liver tissue	115	0.20	13.72	1.39	0.50–0.99

Tab. VIII. Reported studies on zinc concentrations in liver tissue from various sources
* per wet weight ** per dry weight

Author	Source of Material	Zinc concentrations in liver tissue pregnant adult animal		(mMol/kg) Fetus, Newborn	
Hansard et al (13)	cattle	term	0.84	90 d post conception	1.18 *
				180 d post conception	1.84
				270 d post conception	3.09
Apgar (3)	rabbit			newborn	3.50 **
Widdowson et al (47)	human	not pregnant	1.01	20th–40th week of pregnancy	1.71 *
Mutch and Hurley (32)	rat	end of lactation	0.43 ± 0.03		*
Kollmer (22)	rat	virginal	1.41 ± 0.05		**
		pregnant (18 d post conception)	4.28 ± 0.20	18 d post conception	6.78 ± 0.86
		lactating (5 d post natal)	3.66 ± 0.38	5 d post natal	5.35 ± 0.51
Own investigations	rabbit	pregnant (29–30 d post conception)	0.37 ± 0.08	29–30 d post conception	1.35 ± 0.25 *

In the first weeks after birth the capacity of the liver to excret copper in the bile and to synthetize ceruloplasmin increases. Thus the high fetal copper concentrations fall to adult values. It is possible that the growing tissues of the newborn are supplied with some of the copper stored in the liver, however, copper deficiency manifestations are extremely rare in the first months of life.

Very little is known about zinc storage in the fetal liver. WIDDOWSON et al found in human fetal livers near term only slightly higher zinc concentrations than in the liver tissue of adults. **In the course of fetal development the zinc concentration of the liver decreases** [47]. According to HANSARD et al in cattle the zinc concentration in the fetal liver increases steadily during development and near term is 3.7 times higher than the maternal concentration. This storage does not decrease the amount in the maternal organs which also retains zinc during pregnancy [13]. Tab. VIII demonstrates zinc concentrations in maternal and fetal liver tissue as reported by various authors.

For an undisturbed pregnancy a sufficient supply of zinc to the maternal organism is indispensable. Zinc deficient animals have abortions or fetal malformations [1, 8, 11, 15, 16, 17, 42, 45]. The birth process is delayed or made difficult. In the rabbit the nest building instinct is diminished or absent [2, 3].

Our findings confirm that **in the fetal liver of the rabbit zinc is found in concentrations several times higher than those in the maternal liver tissue.** Since there is a positive correlation between fetal and maternal values we assume that the anabolic state of pregnancy causes the similar response of zinc metabolism in fetal and maternal liver. We cannot answer the question as to what causes the fetal liver to store so much more zinc per wet weight than the maternal liver. Since zinc deficient newborns show immediate growth disturbances [32], the zinc contained in the liver in our opinion does not represent storage for later use.

In maternal liver tissue copper and zinc behave alike. There is an association between copper and

zinc concentrations in maternal liver tissue which is not found in the fetal liver tissue. Copper and zinc storage in the fetal liver are independent from each other.

4.2.2 Manganese

This metal is also an essential trace element [5]. Liver arginase [38] and pyruvate carboxylase [30, 40, 44] contain metallic manganese. In mitochondria there is manganese-containing superoxide dismutase. Manganese participates in proteoglycane synthesis by binding of the mucopolysaccharide via a trisaccharide to the polypeptide center [24, 25, 39]. Laboratory animals given manganese deficient feed during a certain phase of pregnancy have a congenital ataxia of the offspring which is caused by a disturbed ossification of the ossicles in the inner ear [14, 15, 18]. Organs rich in mitochondria such as the liver are particularly rich in manganese [5].

In contrast to copper and zinc, storage of manganese in the fetal liver could not be demonstrated by us nor by WIDDOWSON et al [47]. Their mean values (32.8 µMol/kg in the maternal and 27.0 µMol/kg in the fetal liver tissue) agree well with our own findings. Manganese apparently is not stored in the fetal liver. **Because manganese does not pass the placenta newborns have a mild physiological manganese deficiency** and are dependent on the supply with breast milk [19].

4.2.3 Chromium

The biological active form of chromium in the organism is a low-molecular coordination complex consisting of chromium (III), two molecules nicotinic acid and the amino acids glycine, glutamine and cysteine; this has been called glucose tolerance factor (GTF). Nanogram amounts of GTF are co-responsible for the action of insulin. The mechanism has not been explained in more detail nor has the exact structure of GTF [12, 26, 28]. It is assumed that during pregnancy placental GTF transport into the fetus occurs with fetal liver storage of GTF. MERTZ reported that the chromium content is highest in the newborn and declines steadily throughout life [26, 28].

Our results contradict this assumption. **The mean fetal liver concentrations of chromium do not differ from the maternal ones.** WIDDOWSON et al report for human fetus as a mean of 970 nMol/kg and for adults a mean of 865 nMol/kg in the liver tissue. These authors also reported a large scatter of values similar to that observed by us. Thus, we cannot give a statistical distribution of chromium values. After the processing of biological material it is impossible to differentiate between biological active GTF chromium and inorganic chromium. It is possible that in our analyses we covered various chromium compounds of different distribution types. A chromium storage in the fetal rabbit liver did not occur in our material.

4.3 Conclusion

In order to assure a normal pregnancy the fetal organism must be supplied by the placenta with essential metallic elements. A lack of supply leads to malformations. Deposited metals are difficult to mobilize for the organism. This is probably also the case for the high zinc and copper concentrations in the fetal liver.

In the newborn the organism is supplied through breast milk with trace elements. Because ceruloplasmin synthesis increases it is possible that the tissue can be supplied transiently with copper from the liver. In spite of high zinc concentrations in the liver the newborn has not zinc reserves. Zinc deficiency is noticable in the growing experimental animal within a few days. Evidently the newborn does not store manganese and chromium.

This may be important for infant nutrition. The breastfed newborn is adequately supplied with necessary trace elements. **The artificially fed infant is dependent on trace elements in the commercial infant food** [31].

It has not been adequately examined whether the trace element content is sufficient in these preparations. Some of the elements are contained only as "useful contaminations" in these artifical preparations. It is possible that increasing demands for purification result in insufficient presence of trace elements. This would lead to a need for specific additions. It has been assumed that in various areas of the world the supply of zinc and chromium during child years is insufficient.

Summary

Fourty female rabbits at the 29th to 30th day of pregnancy were sacrified and liver tissues taken from the mothers and three fetuses each. About 200 mg of tissues were ashed with nitric acid under high pressure in a closed teflon vessel with a low temperature asher. The residues after ashing were diluted with demineralized water and trace elements, such as Cr, Mn, Cu and Zn, were determined with flameless atomic absorption spectroscopy, except Zn, which was determined by means of the flame technic.
As it is known from other investigations, Cu and Zn have been found in severalfold higher concentrations in fetal liver tissues than in maternal liver tissues. **Liver seems to be a storage organ for Cu and Zn in the fetus.** It is discussed, whether this storage ist purposefull, to provide the newborn with these trace elements.
As far as Cr is concerned, we cannot confirm with other investigations, which showed a storage of Cr in fetal liver tissues. We couldn't find any difference between maternal and fetal liver chromium concentrations in rabbit liver.
The values of manganese were lower in fetal livers than in maternal livers.
These results and the significance of fetal trace element support are discussed.

Keywords: Chromium, copper, fetal development, liver tissue, manganese, rabbits, trace elements, zinc.

Zusammenfassung

Die Spurenelemente Chrom, Mangan, Kupfer und Zink im fetalen Lebergewebe.

Je 200 mg Lebergewebe von 40 graviden Kaninchen zwischen dem 29. und 30. Tag der Schwangerschaft sowie von je 3 der Feten eines Muttertieres wurden mit Salpetersäure unter Druck bei niedriger Temperatur aufgeschlossen. Im Aufschluß wurden die Spurenelemente Cr, Mn, Cu und Zn mit Hilfe der Atomabsorptionsspektrophotometrie bestimmt.
Wie andere Untersucher fanden auch wir stark erhöhte Kupfer- und Zinkkonzentrationen im fetalen Lebergewebe. **Die Leber scheint beim Feten ein Speicherorgan für Kupfer und Zink zu sein.** Es wird diskutiert, ob es sich hierbei um eine Vorratshaltung handelt, um das Neugeborene mit diesen Spurenelementen zu versorgen.
Entgegen anderen Untersuchungen konnten wir eine Speicherung von Cr im fetalen Lebergewebe nicht feststellen. Wir fanden keine Unterschiede zwischen fetaler und mütterlicher Chromkonzentration in der Leber.
Die Mangankonzentrationen lagen im fetalen Lebergewebe niedriger als im mütterlichen.
Diese Ergebnisse und die Bedeutung der Spurenelementversorgung während der Fetalentwicklung werden diskutiert.

Schlüsselworte: Chrom, Fetalentwicklung, Kaninchen, Kupfer, Lebergewebe, Mangan, Spurenelemente, Zink.

Résumé

Les éléments de trace chrome, manganèse, cuivre et zinc dans les tissus hépatiques du foetus

200 mg de tissus hépatiques ont été prélevés sur 40 lapines entre le 29 et 30ème jour de gravidité ainsi que sur trois des foetus de chaque mère. Ces prélèvements ont été dissous à l'acide nitrique sous pression dans un récipient fermé en téflon à basse température. Après dissolutions, les éléments de trace Cr, Mn, Cu et Zn ont été définis à l'aide de la spectroscopie d'absorption atomique.
De même que divers chercheurs l'avaient déjà observé, **nous avons constaté une forte hausse des concentrations de cuivre et de zinc dans les tissus hépatiques des foetus** chez lesquels le foie semble jouer le rôle d'un organe de stockage pour ces éléments. On n'a pas encore réussi à établir, toutefois, s'il s'agit ici d'un stockage destiné à approvisionner le nouveau-né avec ces éléments de trace.

Au contraire d'autres études **nous n'avons pas observé de réserves de Cr dans les tissus hépatiques des foetus** et nous n'avons pas trouvé de différences entre les concentrations de chrome chez les mères et les foetus. Quant au **manganèse, il s'est montré plus concentré dans les tissus hépatiques de la mère que dans ceux du foetus.** La discussion se poursuit sur ces résultats et l'importance de l'approvisionnement en éléments de trace au cours du développement foetal.

Mots-clés: Chrome, cuivre, développement foetal, eléments de trace, lapin, manganèse, tissus hépatiques, zinc.

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